Journal of Thermal Analysis and Calorimetry, Vol. 64 (2001) 713–719

CALORIMETRY TO EVALUATE INCLUSION MECHANISM IN THE COMPLEXATION BETWEEN 2-HYDROXYPROPYL-β-CYCLODEXTRIN AND BARBITURATES IN AQUEOUS SOLUTION

H. Aki, T. Niiya, Y. Iwase and M. Yamamoto

Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Fukuoka University, 8-19-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan

Abstract

Two different types (structures) of inclusion complexes with a 1:1 stoichiometry between barbiturates and 2-hydroxypropyl-β-cyclodextrin (HPCyD) were realized in aqueous solution using isothermal titration calorimetry and molecular dynamics simulation. The first type of complex with a higher association constant was entropy driven and the substituent R_2 was inserted into the HPCyD cavity by hydrophobic interaction. The barbituric acid ring contributed to the second type of complex, which was characterized by large negative values of ΔH and small positive ΔS reflecting van der Waals interaction and/or hydrogen bonding formation between the hetero atoms in the barbituric acid ring and the secondary hydroxyl groups of HPCyD.

Keywords: barbiturates, 2-hydroxypropyl-β-cyclodextrin, inclusion complexes, microcalorimetry

Introduction

In studying inclusion complexes with cyclodextrins, structural information such as stoichiometry, thermodynamics and geometry of the complex are necessary to clarify the complexation mechanism [1, 2]. The values of the association parameter have been determined using a number of physicochemical methods such as spectroscopy, potentiometry, kinetics and the solubility techniques [3]. These methods are mostly based on the typical inclusion complexation that forms only one inclusion type with a 1:1 stoichiometry and a few are based on the stepwise reaction to form two types with 1:1 and 1:2 stoichiometries [4–6]. Nevertheless, there is very little known concerning the multiple types of inclusion complexes with a 1:1 stoichiometry. When the included molecule is not a unique species of an acid/base conjugate solution system, the obtained parameters would not be real and not reflect the contributions of different inclusion types [5, 7]. Isothermal titration microcalorimetry is an ideal analytical method for this purpose, since the values of the association parameters and the enthalpy changes are directly calculated from the data by computer for a single experiment at suitable temperature [8].

1418–2874/2001/ $ 5.00

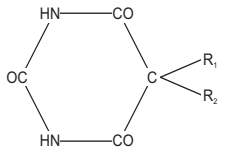
Akadémiai Kiadó, Budapest
Kluwer Academic Publishers, Dordrecht

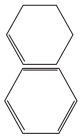
Barbiturates are attractive targets for molecular recognition to form inclusion complex with cyclodextrins. Both their ionized and unionized forms coexist in aqueous solution at physiological pH. In this study, we investigated the effects of substituents at the 5,5-position on the barbituric acid ring on the inclusion types in aqueous solution by calorimetry. Additionally, the geometries of the stable inclusion complexes were presented using molecular dynamics simulations taking water molecules in account.

Materials and methods

Materials

2-Hydroxypropyl-β-cyclodextrin (HPCyD; *MS*=1.0, *m.w.*=~1540) was obtained from Aldrich Chemical Co. Inc. (Milwaukee, USA). Barbital (B) and Phenobarbital (PHB) were purchased from Wako Pure Chemical Ind. Ltd. (Osaka, Jaapn) and pentobarbital (PB), barbituric acid (BA), amobarbital (AB) and cyclobarbital (CB) were from Tokyo Chemical Ind. Co. Ltd. (Tokyo, Japan), and were used without further purification. Barbiturates are shown in scheme. All other materials were of analytical reagent grade.



Barbiturate	R_1	R_2
Barbituric acid	H	
Barbital	CH_2CH_3	CH_2CH_3
Amobarbital	CH_2CH_3	$CH_2CH_2CH(CH_3)_2$
Pentobarbital	CH_2CH_3	$CH(CH_3)CH_2CH_2CH_3$
Cyclobarbital	CH_2CH_3	
Phenobarbital	CH_2CH_3	

Schemes of barbiturates

Microcalorimetry and data analysis

Calorimetric titrations were performed using Thermal Activity Monitor 2270 (Thermometric AB Järfälla, Sweden) and a differential flow microcalorimeter with a twin-cell flow system [9] at 25.0±0.0001°C. All barbiturates and HPCyD were dis-

solved in 1/30 M phosphate buffer solution. A base line was established by flowing the phosphate buffer solution and a barbiturate solution with a constant concentration to correct for the heat of dilution of barbiturates. The solutions of HPCyD at different concentrations (10^{-5} to 10^{-3} M) were titrated sequentially with a constant barbiturate solution. In separate experiments small correction values for the heat of dilution of HPCyD were determined. Assuming that barbiturates as a guest molecule (G) and HPCyD as a host molecule (CD) form different types of inclusion complexes with a 1:1 stoichiometry in aqueous solution as follows:

$$G+CD \leftrightarrow \sum_{i=1}^{n} [G–CD]_i \quad (1)$$

The heat effect of the reaction (Q_r) is proportional to the quantity of the complexes and can be expressed as a function of total concentration of HPCyD:

$$Q_r = [G_t]F_r \sum_{i=1}^{n} \frac{\Delta H_i K_i [CD_f]}{1+K_i [CD_f]} \quad (2)$$

where $[G_t]$ represents the total concentration of barbiturates, $[CD_f]$ the free concentration of HPCyD, F_r the constant flow rate of the calorimetric solutions, and K_i and ΔH_i are the association constant and the enthalpy change for the *i*-th type of inclusion complex $[G–CD]_i$, respectively. A titration curve was obtained by plotting the values of Q_r *vs*. the total concentration of HPCyD added, from which the best fit values of K_i and ΔH_i were calculated simultaneously by computer. The detailed experimental procedures and data analysis were reported elsewhere [8].

Molecular dynamics simulations

Molecular dynamics (MD) simulations for the inclusion complexes of barbiturates with β-cyclodextrin (β-CyD) in aqueous solution were performed using the AMBER program (ver. 4.0) run on a NEC UP4800/65 computer [10]. In the most realistic model of a complex in aqueous solution, the barbiturate and β-CyD were placed in the center of a box containing approximately 380 TIP3P water molecules, with the length of 25, 25 and 21 Å in the *x*, *y* and *z* direction, respectively. The inclusion complex was then subjected to energy minimization to obtain more realistic, low-energy starting structures for MD simulations using the Monte Carlo technique. The MD simulations were equilibrated by 100 ps (Δt=0.001 ps and 100 000 time steps) with SHAKE constraints for hydrogen atoms [11] under conditions of a constant pressure (1 atm) and temperature (293 K).

Results and discussion

Reaction heat of inclusion complexation between barbiturates and HPCyD

The heat of reaction (Q_r) between barbiturates and HPCyD was measured at pH 5.5 and 25°C. The titration data were directly fitted to one- (*i*=1) and two-type (*i*=2) asso-

Table 1 Association constants and thermodynamic parameters of complexation between barbiturates and 2-hydroxypropyl-β-cyclodextrin in pH 5.5 phosphate buffer solution at 25°C

Barbiturates	pK_a	K_1/ 10^4 M^{-1}	$-\Delta G_1$/ kJ mol^{-1}	ΔH_1/ kJ mol^{-1}	ΔS_1/ J mol^{-1} K^{-1}	K_2/ 10^3 M^{-1}	$-\Delta G_2$/ kJ mol^{-1}	$-\Delta H_2$/ kJ mol^{-1}	ΔS_2/ J mol^{-1} K^{-1}
Barbituric acid (BA)	–	0.870	22.5	0.94	72.3	–	–	–	–
Barbital (B)	7.91	0.942	22.7	1.09	72.4	–	–	–	–
Amobarbital (B)	7.94	1.639	24.0	2.50	72.3	1.205	17.6	4.77	43.0
Pentobarbital (PB)	8.11	2.103	24.7	2.74	74.5	3.316	20.1	5.71	51.0
Cyclobarbital (CB)	7.50	5.989	27.3	3.82	78.6	6.364	21.7	15.3	21.4
Phenobarbital (PHB)	7.41	7.245	27.7	4.52	77.9	6.338	21.7	18.5	10.8

The right subscript 1 and 2 indicate the first and the second type of the complexes, respectively

ciation models in Eq. (2). In the case of the complexation of BA and B with HPCyD, the data were fitted to both models. The values of K_1 and K_2 computed from the two-type association model were equal values to that of K obtained from the one-type association model. In contrast, the data for other barbiturates did not fit the one-type association model, but better fitted to the two-type association model. The estimated values of the association constant and the thermodynamic parameters of the first and the second type of complexes are listed in Table 1. The complexation was entropy driven by hydrophobic interaction between barbiturates and the hydrophobic cavity of HPCyD. The association constant was increased in the order of PHB>CB>PB> AB>B>BA.

Influence of pH on inclusion complexation between barbiturates and HPCyD

Barbiturates are essentially unionized at pH 5.5 because of the pK_a values (Table 1), and therefore both the barbituric acid ring and the substituents of barbiturates are hydrophobic.

To evaluate the inclusion structure contributing most to the driving forces and stability of the complexes in aqueous solution, the reaction heat of B, PB and PHB with HPCyD was measured in various pH solutions. The reaction heat effect was decreased at pH values higher than each pK_a, where the barbituric acid ring was ionized. The effects of pH on the thermodynamic parameters for inclusion complexation are shown in Fig. 1.

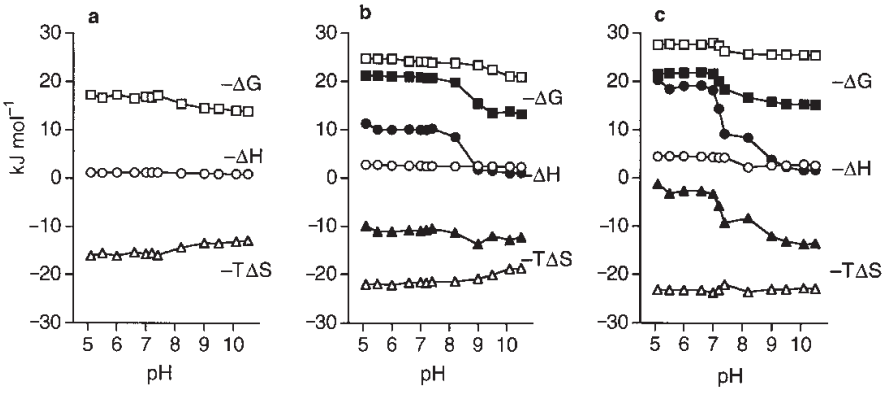


Fig. 1 pH profiles of the thermodynamic parameters for a – B–HPCyD; b – PB–HPCyD and c – PHB–HPCyD complexation in buffer solution. All open symbols represent thermodynamic parameters for the first type of complex and closed symbols are for the second type of complex. □ and ■ $-\Delta G$, o and ● $-\Delta H$, Δ and ▲ $-T\Delta S$

In the first type of inclusion complex with a higher affinity (K_1) for PB and PHB, the thermodynamic parameters were not influenced by pH changes: ΔG_1= –23.5±1.3 kJ mol^{-1}, ΔH_1= –2.52±0.11 kJ mol^{-1} and ΔS_1=70.5±3.9 J mol^{-1} K^{-1} for

PB–HPCyD complex and $\Delta G_1 = -26.0 \pm 1.0$ kJ mol^{-1}, $\Delta H_1 = -3.73 \pm 0.86$ kJ mol^{-1}, and $\Delta S = 77.7 \pm 1.5$ J mol^{-1} K^{-1} for PHB–HPCyD complex. The small negative values of ΔH_1 and the large positive ΔS_1 indicate hydrophobic interaction, reflecting complexation between the hydrophobic cavity of HPCyD. In the second type of inclusion complexation with lower affinity (K_2), there was a large inflection in the values of ΔG_2 and ΔH_2 around the pK_a value of each barbiturate. The large negative values of ΔH_2 and the small positive ΔS_2 in the complexation of the unionized barbiturates with HPCyD at pH lower than the pK_a values were owing to the van der Waals interaction and/or hydrogen bonding formation. The values of K_2 were markedly decreased below 10^3 M^{-1} when the barbiturates were ionized over pH 8.0. It was suggested that the interactions between the ionized barbiturates and HPCyD were not strong enough to form the inclusion complex.

The thermodynamic parameters of B and BA with only one type of inclusion complex were considerably smaller than those of any other barbiturates and not influenced by pH changes: $\Delta G = -15.9 \pm 1.3$ kJ mol^{-1}, $\Delta H = -1.01 \pm 0.13$ kJ mol^{-1}, and $\Delta S = 49.9 \pm 4.1$ J mol^{-1} K^{-1} for B–HPCyD complex formation. B and BA would be loosely bound to HPCyD with a low affinity.

Mechanism of inclusion complexation between barbiturates and HPCyD

Calorimetric results show that two different types of the inclusion complexes with a 1:1 stoichiometry of unionized barbiturates with HPCyD, except B and AB, are formed by penetration of the substituent R_2 or the barbituric acid ring into the cavity of HPCyD in aqueous solution. To clarify the structures of the inclusion complexes, the unsubstituted β-cyclodextrin (β-CyD) was used as a host molecule in MD simulation. The calculations started from first initial geometries of the inclusion complexes in aqueous solution and those behaviours were simulated for 100 ps [12]. As the results, Fig. 2 shows the snapshots of the stable equilibrium geometries for B–β-CyD, PB–β-CyD and PHB–β-CyD inclusion complexes at 100 ps. In the Type I simulation, the substituent R_2 of barbiturates was initially placed in the β-CyD cavity, whereas other groups were situated at the rim of the secondary hydroxyl side of β-CyD. The barbituric acid ring was initially located in the center of the cavity in the Type II, whereas other groups were also situated at the rim of the secondary hydroxyl side. Energetically, Type I was more stable than Type II at equilibrium.

In the chemical structure of HPCyD, 2-hydroxypropyl groups are linked to the primary side-chains of β-CyD. In the first type of inclusion complex with K_1 driven by the hydrophobic interaction, the barbiturate substituent R_2 initially was inserted into the cavity from the secondary hydroxyl side of HPCyD to form Type I complexes, which were very stable in aqueous solution at various pH values. In the second type of inclusion complex with K_2, the unionized form of barbituric acid ring was inserted into the cavity of HPCyD to form Type II complexes. This inclusion type could provide certain stability to the complexes by van der Waals interaction and/or hydrogen bonding formation by carbonyl groups on the barbituric acid ring rather than hydrophobic interaction. In both types, the ethyl-side chain of substituent R_1 re-

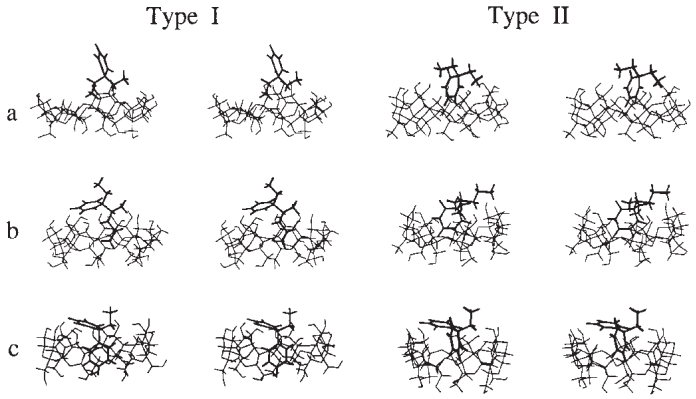


Fig. 2 Stereoscopic views of the two types of inclusion complexes for a – B–β-CyD; b – PB–β-CyD and c – PHB–β-CyD in aqueous solution. Type I and Type II show the first and the second type of the inclusion complexes, respectively. All inclusion types are represented as the final geometries through 100 ps simulation. The thick and thin lines represent guest and host molecules, respectively

mained outside of the HPCyD cavity. On the other hand, B and AB were loosely bound to HPCyD with K of 10^3 M^{-1} to form lid-type supramolecular complexes (Type II) as shown in Fig. 2.

* * *

This work was financially supported in part by Hayashi Memorial Foundation for Female Natural Scientists in Japan to H. Aki.

References

1 E. Junquera and E. Aicart, J. Pharm. Sci., 88 (1999) 626.
2 Y. Inoue, T. Hakushi, Y. Liu, L.-H. Tong, B.-J. Shen and D.-S. Jin, J. Am. Chem. Soc., 115 (1993) 475.
3 K.-H. Frömming and J. Szejtli, Cyclodextrins in Pharmacy, Kluwer 1992, p. 45.
4 A. Grade, V. Rüdiger, H.-J. Schneider and K. A. Connors, J. Pharm. Sci., 86 (1997) 236.
5 P. Bouderille and J.-L. Burgot, J. Pharm. Sci., 84 (1995) 1083.
6 A. M. Myles, D. J. Barlow, G. France and M. J. Laurence, Biochim. Biophys. Acta, 27 (1994) 27.
7 E. E. Sideris, G. N. Valsami, M. A. Koupparis and P. E. Macheras, Pharm. Res., (1992) 1568.
8 H. Aki, T. Niiya, Y. Iwase and M. Yamamoto, Thermochim. Acta, 308 (1998) 115.
9 M. Yamamoto and H. Aki, J. Biochem. Biophys. Methods, 16 (1994) 1912.
10 P. K. Weiner and P. A. Kollman, J. Comp. Chem., 2 (1981) 287.
11 J. P. Rychaert, G. Ciccotti and H. J. C. Berendsen, Comput. Phys., 23 (1997) 327.
12 H. Aki, T. Haraguchi, T. Niiya, Y. Goto and M. Yamamoto, Yakugaku Zassi (J. Pharm. Soc., Japan), 116 (1996) 803.